ABSTRACT OF THE DISCLOSURE

Oligonucleotide-fluorophore-quencher conjugates wherein the fluorophore moiety has emission wavelengths in the range of about (300) to about (800) nm, and or where the quencher includes a substituted 4-(phenyldiazenyl)phenylamine structure provide improved signal to noise ratios and other advantageous characteristics in hybridization and related assays. The oligonucleotide-fluorophore-quencher conjugates can be synthesized by utilizing novel phosphoramidite reagents that incorporate the quencher moiety based on the substituted 4-(phenyldiazenyl)phenylamine structure, and or novel phosphoramidite reagents that incorporate a fluorophore moiety based on the substituted coumarin, substituted 7-hydroxy-3H-phenoxazin-3-one, or substituted 5,10-dihydro-10[phenyl]pyrido[2,3-d;6,5-d']dipyrimidine-2,4,6,8-(1H, 3H, 7H, 9H, 10H)-tetrone structure. Oligonucleotide-fluorophore-quencher-minor groove binder conjugates including a pyrrolo[4,5-e]indolin-7-yl}carbonyl)pyrrolo[4,5-e]indolin-7-yl]carbonyl}pyrrolo[4,5-e]indolin-7-caroxylate (DPI_3) moiety as the minor groove binder and the substituted 4-(phenyldiazenyl)phenylamine moiety as the quencher, were synthesized and have substantially improved hybridization and signal to noise ratio properties.